TCDD-Induced Changes in Rat Liver Microsomal Enzymes

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a contaminant of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), is extremely toxic (*1*), although the mechanism of toxicity is not known. Other papers presented at this conference cover the spectrum of environmental and health hazards of chlorinated dibenzodioxins and dibenzofurans. It should suffice to say here that these compounds are teratogens (*2–4*) in rodents, and the extensive use of 2,4,5-T, especially in Vietnam, has focused concern on their potential health hazards. Recently TCDD was shown to be an inducer of δ-aminolevulinic acid synthetase in the chick embryo (*5*) and also to decrease hexobarbital sleeping times in rats (*6*). These reports prompted us to investigate the effects of sublethal doses of TCDD on activities of hepatic microsomal and mitochondrial enzymes. The microsomal enzymes include components that are involved in the detoxication of foreign compounds and the regulation of many endogenous compounds such as the steroid hormones (*7*). Microsomal constituents and activities investigated in this study were: cytochrome P-450, cytochrome b_5, benzpyrene hydroxylation, aniline hydroxylation, aminopyrine demethylation, benzphetamine demethylation, ethylmorphine demethylation, NADPH cytochrome c reductase, β-glucuronidase, and UDP glucuronyltransferase. We also monitored possible changes in oxidative phosphorylation rates in rat liver mitochondria to determine if the toxic action of TCDD could be related to disruptions in bioenergetic pathways.

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Materials and Methods

Animals

Male and female rats (Charles River, CD strain) were used in these experiments. On the day of treatment rats weighed approximately 200 g (males 6 weeks old, females 8 weeks old). TCDD was administered as a single oral dose in 0.5 ml acetone–corn oil, and controls received 0.5 ml acetone–corn oil (*8*).

Preparation of Subcellular Fractions

Rats were killed by cervical dislocation, and approximately 4.0 ml blood was immediately drawn from the dorsal aorta. Livers were removed, minced, and homogenized in 1.15% KCl buffered with 0.02*M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.5, at 5°C to make a 20% (w/v) mixture. Homogenization was accomplished by using 6 strokes in a motor-driven Potter-Elvehjem homogenizer. Nuclei and cell debris were removed by centrifugation at 670*g* for 10 min and mitochondria re-

moved by centrifugation of the 670*g* supernatant at 10000*g* for 15 min. Microsomes were pelleted by centrifugation of the post-mitochondrial supernatant at 105000 *g* for 70 min, washed once with HEPES–KCl buffer, and finally resuspended in HEPES so that 1.0 ml of microsomal suspension contained material from 0.5g liver (wet weight). Smooth- and rough-surfaced endoplasmic reticulum (SER and RER) fractions were prepared by homogenizing chopped liver sections in 0.25*M* sucrose (pH 7.0) and preparing the microsomal subfractions on discontinuous sucrose gradients by the procedure of Gram et al. (*9*), 10 ml of post-mitochondrial supernatant and 12 ml of 1.3-*M* sucrose being used. Liver mitochondria were prepared by the procedure of Nelson et al. (*10*) and resuspended in 0.25*M* sucrose so that 2.0 ml of suspension contained mitochondria from 3.0g liver (wet weight).

Assay Methods

Cytochrome P-450 was measured by its carbon monoxide difference spectra in an ACTA III spectrophotometer following reduction with dithionite, and cytochrome b_5 was measured by its difference spectra following reduction with NADH (*11*). For the determination of *in vitro* microsomal hydroxylation of aniline and demethylation of aminopyrine and ethylmorphine, the previously described incubation medium (*12*) was employed, with the exception that HEPES buffer was used instead of Tris buffer. Concentrations of substrates were: 3.5*mM* aniline, 2.5*mM* ethylmorphine, or 2.5*mM* aminopyrine in 3.0 ml incubation medium. Enzyme reactions were started by the addition of 1.5–2.0 mg microsomal protein. Benzpyrene (BP) hydroxylation and benzphetamine demethylation rates were determined by using the incubation medium of Hook et al. (*13*). There was essentially no difference when an NADPH regenerating system (*12*) or saturating concentrations of NADPH (3.1 *mM*) were used in the incubation medium. Aniline hydroxylation was quantified by the method of Kato and Gillette (*14*). Formaldehyde released by the demethylation of benzphetamine, aminopyrine, and ethylmorphine was measured by the Nash reaction (*15*). BP hydroxylation was measured by the fluorescence method of Wattenberg et al. (*16*). NADPH cytochrome c reductase was measured by the reduced cytochrome c peak at 550 nm (*17*). β-Glucuronidase was determined by the modified method (*18*) of Talalay et al. (*19*), phenolphthalein β-D-glucuronide being used as the substrate. *p*-Nitrophenol glucuronyltransferase was determined spectrophotometrically (*20*) by using 0.9*mM* p-nitrophenol, 0.8*mM* UDPGA, 10*mM* $MgCl_2$, and Triton X-100-treated microsomes (*21*). After 3 min incubation, the reaction was stopped by the addition of 5.0 ml glycine buffer, pH 10.4 (*21*). Experimental data for glucuronyltransferase were similar in all cases whether activity was measured by *p*-nitrophenol disappearance (*20*) or *p*-nitrophenyl β-D-glucuronide appearance at 312 nm (*22*). Oxidative phosphorylation rates in isolated liver mitochondria were measured polarographically with a Clark oxygen electrode. The reaction mixture contained 120*mM* KCl, 12*mM* substrate (succinic acid), 8*mM* $MgCl_2$, 5*mM* K_2HPO_4, 10.0*mM* ADP and 20*mM* glyclglycine buffer (pH 7.4). The total volume was 1.6 ml and the temperature was maintained at 30°C. Oxygen content in the vessel was calibrated by using NADH. Microsomal and mitochondrial protein contents were determined by the method of Lowry et al. (*23*).

Results and Discussion

Time-Course Studies

Male rates were administered TCDD as a single oral dose at 5 or 25 μg/kg, and hepatic microsomal enzyme activities and cytochrome contents were measured 1, 3, 9, 16, and 28 days after treatment. The purposes of this study were to determine whether TCDD affected microsomal enzyme activities and, if so, to determine the time–course alterations in enzyme activities. The LD_{50} value for TCDD is approximately 100 μg/kg (John Moore, personal communication), and no lethality of TCDD to male rats was observed at 5 or 25 μg/kg in test animals.

Aniline hydroxylation—Time-course effects of TCDD on aniline hydroxylation are presented in Figure 1. Aniline hydroxylation is expressed as nanamoles *p*-aminophenol formed per minute per milligram protein. Enzyme activity was slightly but not significantly enhanced at day 1. By day 3, hydroxylation rates were increased over 100%, and the same level of induction was observed through day 16. After day 16, enzyme activities began to return to control values, although aniline hydroxylation was still significantly elevated 38 days after treatment with 25 μg/kg TCDD.

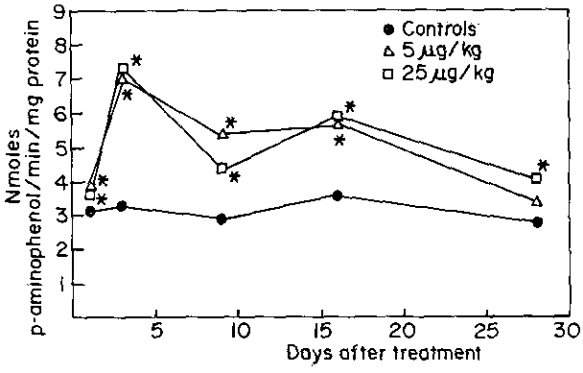

FIGURE 1. Time-course effects of a single oral dose of TCDD on liver microsomal aniline hydroxylation. An asterisk indicates that values are significantly different from controls at $P < 0.05$. $N = 3$ male rats.

Aminopyrine demethylation—The effects on aminopyrine demethylation were opposite those observed for aniline hydroxylation (Fig. 2). Specific enzyme activity was decreased approximately 30% at days 3, 9, and 16 by the 25 μg/kg dose. Values were essentially unchanged at the lower dose.

Cytochrome P-450 and b_5—Cytochrome P-450 was increased by 40% at day 1 and cytochrome b_5 was unchanged at day 1, while at day 3 contents of both microsomal cytochromes were elevated although P-450 was increased more than b_5 (Figs. 3 and 4). Nine days after treatment P-450 and b_5 were both increased by 60%. The lag period in b_5 effects compared to P-450 might be related to the slower turnover rate of cytochrome b_5 (*24*). Thirty-eight days after TCDD treatment (25 μg/kg), b_5 content was increased by 60% and P-450 was increased by 40%. Increased P-450 content was asso-

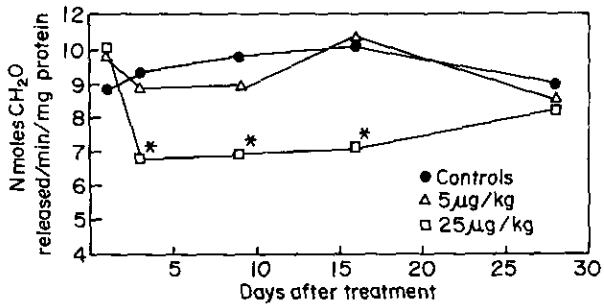

FIGURE 2. Time-course effects of a single oral dose of TCDD on liver microsomal aminopyrine demethylation. An asterisk indicates that values are significantly different from controls at $P < 0.05$. $N = 3$ male rats.

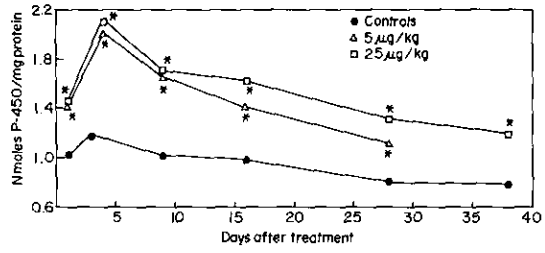

FIGURE 3. Time-course effects of a single oral dose of TCDD on liver microsomal cytochrome P-450. An asterisk indicates that values are significantly different from controls at $P < 0.05$. $N = 3$ male rats.

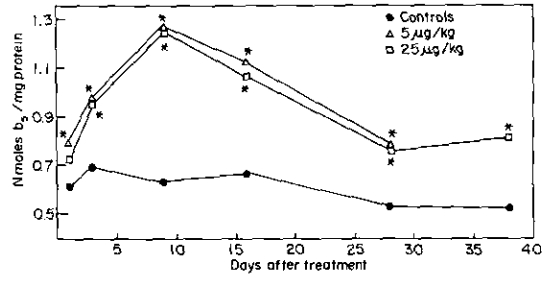

FIGURE 4. Time-course effects of a shingle oral dose of TCDD on liver microsomal cytochrome b_5. An asterisk indicates that values are significantly different from controls at $P < 0.05$. $N = 3$ male rats.

ciated with increased oxidative hydroxylation and decreased oxidative demethylation. This induction pattern is similar to that observed for 3-methylcholanthrene (*25, 26*). However, TCDD appears not to shift the peak in the carbon monoxide difference spectra from 450 nm to 448 nm such as occurs with 3-methylcholanthrene induction (*26*). TCDD obviously cannot be considered a phenobarbital-type inducer, which is characterized by increased P-450 content, increased hydroxylation activity, and increased oxidative *N*-demethylation (*27*).

UDP glucuronyltransferase—Effects on glucuronyltransferase were the most striking observed in the time–course study (Fig. 5). Following TCDD treatment at 25 μg/kg enzyme activity was enhanced by 51% on day 1, 162% on day 3, 565% on day 9, 636% on day 16, 154% on day 28, and 162% on day 38. Levels of increases were slightly less at the 5 μg/kg dose compared to the 25 μg/kg dose, and time–course effects were similar. Increased glucuronlytransferase activity was not associated with changes in k_m values for substrate (*p*-nitrophenol, 0.26-*mM*) or co-factor (UDPGA, 0.58*mM*) (*28*). V_{max} in control animals was 126 nmole *p*-nitrophenol conjugated/min-mg protein compared to 539 in microsomal preparations from TCDD-treated rats (*28*). UDP glucuronyltransferase activity is phospholipid-dependent (*29, 30*), and microsomal cholesterol has been theorized to function in the maintenance of endoplasmic reticulum structure (*31*). However, TCDD elevation of glucuronyltransferase activity does not appear to be related to alterations in total microsomal phospholipid and cholesterol levels (*28*), although individual microsomal phospholipids have not been quantified following TCDD treatment. Divalent cations and detergents are *in vitro* activators of microsomal glucuronyltransferase (*21*) and the effects of TCDD might be related to detergentlike actions on the endoplasmic reticulum or to mobilization of endogenous magnesium or other stimulatory divalent cations. However, the magnitude of the effect on glucuronyltransferase was the same whether glucuronyltransferase was measured in the presence or absence of Mg^{+2} or Triton X-100 (*28*). These data suggest that TCDD effects on glucuronyltransferase are not related to morphological alterations in endoplasmic reticulum structure, although this possibility has not been excluded. However, at this stage it appears that the possibilities that best fit the experimental data are related to increased enzyme synthesis or decreased degradation rates. TCDD induction of ALA synthetase in the chick embyro was blocked by cycloheximide (*5*) but data from protein-synthesis inhibition experiments would be difficult to obtain in rats due to the lag period in TCDD induction and the rapid toxicity of most antimetabolities. Elevation of glucuronyltransferase occurred in kidney microsomes as well as liver microsomes (*28*), although distribution studies have demonstrated that liver accumulates OCDD-Cl^{36} equivalents at much higher levels than kidneys (*32*). TCDD did not affect glucuronyltransferase when added directly to the incubation medium at 10^{-6} *M*.

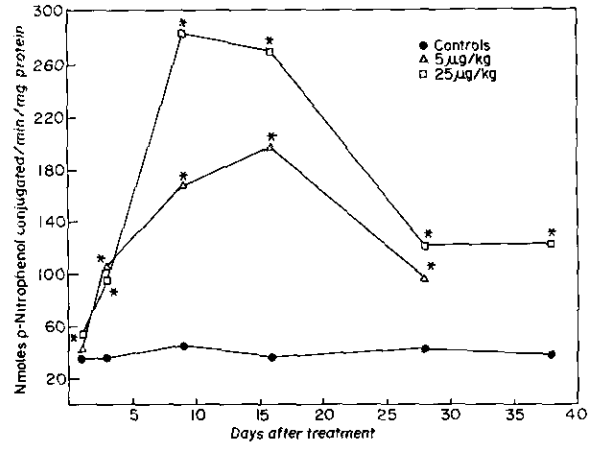

FIGURE 5. Time–course effects of a single oral dose of TCDD on liver microsomal UDP glucuronyltransferase. An asterisk indicates that values are significantly different from controls at $P < 0.05$. $N = 3$ male rats.

Microsomal protein—Since enzyme activities and cytochrome contents were measured on a per-milligram protein basis, it was of importance to measure time–course effects on microsomal protein contents. Previous

reports show that chronic exposures to chlorinated triphenyl compounds markedly increased microsomal protein contents (*33*). Hepatic ultrastructural studies of TCDD-treated rats revealed general proliferation of RER and increases in SER in specific hepatic areas (*34*). In our studies, microsomal protein contents were not significantly changed till 28 days after a single TCDD treatment when levels were enhanced by 60% (Table 1). Therefore, enzyme activities per gram liver had essentially the same relative values between control and TCDD-treated rats as when enzyme activities were calculated per milligram protein.

Other enzymes—NADPH cytochrome c reductase and β-glucuronidase were not affected at either dose or at any period during the time–course experiment.

Liver function—Possible hepatotoxicity was monitored by serum orinthine transcarbamylase activities (*35*). Results show that after rats received 5 or 25 μg TCDD/kg there were no indications of liver damage at any time period.

Dose-Response Studies

Dose–response relationships were examined in both male and female rats to obtain information concerning sex differences of hepatic microsomal responses to TCDD and to determine what is the lowest dose that results in induction of microsomal enzymes. Enzymes investigated in these studies included those used in the time–course experiment plus BP hydroxylation, benzphetamine demethylation, and ethylmorphine demethylation. These enzymes were added so that a more extensive comparison could be made on the different effect of TCDD on oxidative hydroxylations and demethylations. Single doses of TCDD at 0.2, 1.0, 5.0, and 25 μg/kg were used, and microsomal enzymes assayed 3 days after treatment.

Table 2. Changes in activities of male rat liver microsomal enzymes following a single oral dose of TCDD.[a]

Enzyme	Relative change from control values (100)[b]			
	TCDD, 0.2 μg/kg	TCDD, 1.0 μg/kg	TCDD, 5.0 μg/kg	TCDD, 25.0 μg/kg
Cytochrome P–450	119	162 [c]	184 [c]	193 [c]
Cytochrome b_5	101	139 [c]	167 [c]	195 [c]
Aminopyrine demethylation	103	90	86 [c]	72 [c]
Benzphetamine demethylation	101	68 [c]	70 [c]	59 [c]
Ethylmorphine demethylation	101	79	77	—
Aniline hydroxylation	124 [c]	160 [c]	202 [c]	198 [c]
Benzpyrene hydroxylation	102	163 [c]	163 [c]	467 [c]
Glucuronyl-transferase	138 [c]	167 [c]	385 [c]	471 [c]
Protein	112 [c]	113	105	126 [c]

[a] Rats were killed 3 days after TCDD treatment at various TCDD dose levels. Each value is derived from four animals.

[b] Control values ± S.D. were: cytochrome P–450, 0.66 ± 0.08 nmole/mg protein; cytochrome b_5, 0.40 ± 0.01 nmole/mg protein; aminopyrine demethylation, 9.1 ± 0.4 nmole formaldehyde released/min–mg protein; benzphetamine demethylation, 8.1 ± 1.5 nmole formaldehyde released/min–mg protein, ethylmorphine demethylation, 15.9 ± 3.0 nmole formaldehyde released/min–mg protein; aniline hydroxylation, 3.1 ± 0.5 nmoles *p*-aminophenol formed/min–mg protein; glucuronyltransferase, 49.1 ± 0.8 nmoles *p*-nitrophenol conjugated/min–mg protein; microsomal protein, 25.3 ± 2.7 mg/g liver.

[c] Significantly different from controls at $P<0.05$.

Table 1. Time–course effects of a single oral dose of TCDD on microsmal protein.[a]

Dose, TCDD, μg/kg	Microsomal protein after TCDD treatment, mg/g liver				
	1 day	3 days	9 days	16 days	28 days
0	16.8 ± 1.7	16.8 ± 0.5	18.1 ± 3.0	19.3 ± 1.8	17.6 ± 2.6
5	18.4 ± 3.3	19.0 ± 5.4	21.8 ± 2.2	21.2 ± 0.9	28.2 ± 3.1 [b]
25	17.8 ± 3.6	23.1 ± 3.2 [b]	19.1 ± 1.2	20.6 ± 3.5	29.4 ± 3.5 [b]

[a] Values at various times after TCDD treatment; $N = 3$ male rats.

[b] Significantly different from controls at $P < 0.05$.

Males—Data on the effects of TCDD on male rat liver microsomal enzymes are presented in Tables 2 and 3. Increases in cytochrome P-450 and b_5 contents were not evident until animals received 1.0 μg/kg and the level of induction was dose-dependent up to 25 μg/kg. Activities of oxidative demethylation enzymes for all three substrates were decreased in a dose-dependent manner. Aniline hydroxylation was enhanced by 0.2 μgTCDD/kg (24%) and maximum increases occurred after 5.0 μg TCDD/kg (102%). Induction of BP hydroxylation was similar in magnitude to aniline hydroxylation at the three lower doses but after male rats received 25 μg TCDD/kg, BP hydroxylation was increased by 300% and aniline hydroxylation by 100%. Glucuronyltransferase was increased by 38, 67, 285, and 371% at the four doses from the lowest to highest, respectively. When enzyme activities per nmole cytochrome P-450 were calculated, oxidative demethylation values of the three substrates tested were significantly decreased (50–70%) at TCDD doses of 1.0 μg/kg or greater (Table 3). Hydroxylation values per P-450 unit were essentially unchanged, with the exception that BP hydroxylation per unit of P-450 increased by approximately 100% following a dose of 25 μg TCDD/kg.

Table 4. Changes in activities of female rat liver microsomal enzymes following a single oral dose of TCDD.[a]

Enzyme	Relative change from control values (100)[b]		
	TCDD, 0.2 μg/kg	TCDD, 1.0 μg/kg	TCDD, 5.0 μg/kg
Cytochrome P-450	126[c]	153[c]	196[c]
Cytochrome b_5	122[c]	131[c]	158[c]
Aminopyrine demethylation	120[c]	131[c]	120[c]
Benzphetamine demethylation	115	112	118
Benzpyrene hydroxylation	783[c]	1225[c]	1403[c]
Glucuronyltransferase	257[c]	506[c]	487[c]
Protein	94[c]	108[c]	115[c]

[a] Rats were killed 3 days after TCDD treatment at various TCDD dose levels. Each value derived from four animals.

[b] Enzyme activities were expressed as indicated in Table 2. Control values ± S.D. were; cytochrome P-450, 0.45 ± 0.06 nmole/mg protein; cytochrome b_5, 0.36 ± 0.03 nmole/mg protein; aminopyrine demethylation, 4.4 ± 0.3 nmole formaldehyde/min–mg protein; benzphetamine demethylation, 2.34 ± 0.45 nmole formaldehyde/min–mg protein; benzpyrene hydroxylation, 0.06 ± 0.01 nmole/mg–min protein; glucuronyltransferase 23.9 ± 5.4 nmole/min–mg protein; and microsomal protein (21.0 ± 5.0 mg/g liver).

[c] Significantly different from controls at $P<0.05$.

Table 3. Effect of TCDD on mixed function oxidase activity per cytochrome P-450 unit in male rat liver microsomes

Enzyme	Activity, nmole substrate metabolized/nmole P-450				
	0	TCDD, 0.2 μg/kg	TCDD, 1.0 μg/kg	TCDD, 5.0 μg/kg	TCDD, 25.0 μg/kg
Aminopyrine demethylation	13.7 ± 1.0	12.4 ± 1.5	7.6 ± 0.8[b]	6.4 ± 0.7[b]	4.6 ± 0.3[b]
Benzphetamine demethylation	12.2 ± 1.8	10.9 ± 2.3	5.1 ± 0.9[b]	4.6 ± 0.4[b]	—
Ethylmorphine demethylation	23.8 ± 1.5	21.3 ± 3.8	11.7 ± 2.2[b]	10.0 ± 1.6[b]	—
Aniline demethylation	3.4 ± 0.2	3.2 ± 0.7	3.8 ± 0.1	3.7 ± 0.3	3.5 ± 0.6
Benzpyrene hydroxylation	0.80 ± 0.14	0.72 ± 0.17	0.80 ± 0.03	0.71 ± 0.07	1.95 ± 0.20[b]

[a] Rats sacrificed 3 days after TCDD treatment at various dose levels. $N = 4$ male rats.

[b] Significantly different from controls at $P<0.05$.

Table 5. Effect of TCDD on mixed function oxidase activity per cytochrome P-450 unit in female rat liver microsomes.[a]

Enzyme	Activity, nmole substrate metabolized/nmole P-450			
	0	TCDD, 0.2 μg/kg	TCDD, 1.0 μg/kg	TCDD, 5.0 μg/kg
Aminopyrine demethylation	9.9 ± 1.5	9.3 ± 0.2	8.4 ± 0.6	6.1 ± 1.0[b]
Benzphetamine demethylation	5.1 ± 1.0	4.7 ± 0.6	3.7 ± 0.7	3.1 ± 0.4[b]
Ethylmorphine demethylation	7.3 ± 1.4	6.4 ± 0.5	4.4 ± 0.2[b]	3.4 ± 0.7[b]
Benzpyrene hydroxylation	0.13 ± 0.05	0.8 ± 0.1[b]	1.0 ± 0.21[b]	0.96 ± 0.16[b]

[a] Rats sacrificed 3 days after TCDD treatment. $N = 4$ male rats.
[b] Significantly different from controls at $P < 0.05$.

Females—Data on the effects of TCDD on female microsomal enzymes are presented in Tables 4 and 5. Female rats were more susceptible to TCDD induction of BP hydroxylation and glucuronyltransferase than male rats (Table 4). This sex difference was quite evident following a dose of 0.2 μg/kg; male liver microsomal glucuronyltransferase increased 38% and female liver microsomal glucuronyltransferase increased 157%, male BP hydroxylation increased 2% and female BP hydroxylation increased 683%. In control animals, BP hydroxylation rates were eight times greater in liver microsomes from males compared to females, but in TCDD-treated rats activities of liver microsomal BP hydroxylase were approximately the same in both sexes. Glucuronyltransferase activity of liver microsomes from males was twice that of females in controls, but in TCDD-treated rats (0.2 μg/kg) activities were higher in microsomes from females than males. Oxidative demethylation activites were two to four times as high in microsomes from control males compared to females. Female hepatic microsomal *N*-demethylations were slightly increased by TCDD, whereas corresponding enzyme activities in male hepatic microsomes were decreased although *N*-demethylation rates were still higher in TCDD-treated males compared to TCDD-treated females at all dose levels. Since maximum elevation of P-450 in females, as in males, was approximately 100%, *N*-demethylations per unit P-450 were decreased, but in general the observed decrease in *N*-demethylations per P-450 unit in females was not as much as that in males.

These data demonstrate that TCDD markedly increases activity of some microsomal enzymes, particularly glucuronyltransferase and BP hydroxylase, and that female rats are more susceptible to action of TCDD than males. Increases in activity of microsomal enzymes after a dose of 0.2 μg TCDD/kg is quite significant in comparison to doses required for effects by other inducing agents. TCDD is approximately 100,000 times more potent an inducing agent than phenobarbital or 3-methylcholanthrene on a μg/kg basis in rats. In addition to our studies, Hook et al. (*36*) have shown that a single TCDD dose of 0.2 μg/kg to female rats increased biphenyl 2-hydroxylation by approximately 900% and biphenyl 4-hydroxylation by approximately 100%. Therefore, an oral dose of 40 ng TCDD to 200 g rats markedly increases activity of microsomal enzymes. Norback et al. (*32*) report that 95% of labeled TCDD is excreted in the feces following oral administration. Therefore, it appears that only a small portion of the administered TCDD reaches the liver, although it is possible that much of the fecal radioactivity has been added via biliary excretion and that intestinal absorption rates might vary with dose. ALA synthetase activity was increased in chick embryos by extremely low concentrations of TCDD (*5*), but in the same group of rats used in our studies, hepa-

tic ALA synthetase was not changed (*37*) by doses 50 times that needed to increase UDP glucuronyltransferase, benzpyrene hydroxylation, and biphenyl 2-hydroxylation. The extreme sensitivity of microsomal enzymes to TCDD body burdens suggest that alterations in activities of these enzymes could be related to the toxic action of TCDD and its teratogenic effects by disrupting normal steriod regulation. Adrenal size is not enlarged in TCDD-treated rats (*38*), indicating that if steriod excretion is enhanced the compensatory feedback mechanism controlling steroid synthesis may not be operative.

Effects of TCDD on SER and RER

Effects of TCDD on the distribution of microsomal components in SER and RER of male rats are summarized in Table 6. SER to RER ratios were decreased in all parameters tested following TCDD treatment (25 μg/kg). *N*-Demethylation ratios (SER:RER) were approximately 2.4 in controls compared to 0.7 in treated rats. Specific demethylation activities were decreased by 75% in SER and were essentially unchanged in RER. BP hydroxylation was elevated in both SER and RER, but induction was greater in RER resulting in decreased SER:RER from 1.77 to 1.19. Glucuronyltransferase was also markedly increased in both subfractions (SER, 200%; RER, 300%) and SER:RER decreased from 0.56 to 0.37. Microsomal protein also exhibited decreased SER:RER following TCDD treatment although the change was not significant. These changes in SER:RER ratios are similar to those seen after 3-methylcholanthrene treatment (*26*). Ultrastructural studies revealed overall RER proliferation and SER proliferation in isolated regions of rat liver hepatocytes (*34*).

Effects on Oxidative Phosphorylation

The gradual wasting of animals that precedes death in TCDD-exposed animals suggested that toxicity might be an expression of bioenergetic disturbances. However, oxidative phosphorylation rates in isolated rat liver mitochondria from TCDD-treated rats (5 or 25 μg TCDD/kg) did not significantly differ from those in controls (Table 7). Parameters investigated were state 3 respiration, state 4 respiration, respiratory control (R.C.) (state 3/state 4) and ADP:O. The only difference observed between control and treated rats was greater uncoupling rates of the treated group on storage at 4° C.

Table 6. Submicrosomal distribution of male rat liver microsomal enzymes following a single oral dose of TCDD (25 μg/kg).[a]

Enzyme[b]	Control			TCDD		
	SER	RER	SER:RER	SER	RER	SER:RER
Aminopyrine demethylation, nmole HCHO/min–mg	9.3 ± 1.0	4.0 ± 0.6	2.34	2.6 ± 0.1	3.6 ± 0.7	0.73[c]
Benzphetamine demethylation, nmole HCHO/min–mg	9.1 ± 1.7	3.8 ± 0.4	2.38	2.1 ± 0.1[c]	3.5 ± 0.4	0.61[c]
Benzpyrene hydroxylation, nmole/min–mg	0.46 ± 0.09	0.26 ± 0.08	1.77	2.4 ± 0.3[c]	2.0 ± 0.2[c]	1.19[c]
Glucuronyl-transferase, nmole/min–mg	69.8 ± 17.2	124.5 ± 31.9	0.56	205.8 ± 22.8[c]	556.7 ± 87.9[c]	0.37[c]
Protein, mg/g liver	5.9 ± 0.5	12.8 ± 0.6	0.46	6.2 ± 0.4	16.8 ± 1.8[c]	0.37[c]

[a] Rats were killed six days after TCDD treatment. $N = 4$ male rats.
[b] Enzyme activities expressed as outlined in footnotes b to Table 2.
[c] Significantly different from controls at $P<0.05$.

Table 7. Effects of a single oral dose (25 μg/kg) of TCDD on oxidative phosphorylation rates in rat liver mitochondria.[a]

Time after treatment, days	State 3 respiration, ng-atom O/min–mg protein	State 4 respiration, ng-atom O/ min–mg protein	R.C.	ADP:O
Controls	138 ± 14	34 ± 5	4.06	1.85 ± 0.06
1	133 ± 7	31 ± 3	4.33	1.88 ± 0.04
3	159 ± 22	37 ± 7	4.27	1.71 ± 0.09
6	145 ± 4	37 ± 2	3.92	1.73 ± 0.08
9	127 ± 6	31 ± 2	4.10	1.73 ± 0.14
16	140 ± 13	36 ± 1	3.89	1.78 ± 0.21
28	146 ± 5	37 ± 3	3.95	1.75 ± 0.07

[a] Succinate used as the substrate; each value (mean ± S.D.) derived from an average of four male rats.

Summary

Male or female rats were administered a single oral dose of TCDD at 0.2, 1.0, or 5.0 μg/kg, and activities of hepatic microsomal enzymes were monitored three days after treatment. Our data demonstrate that TCDD has an extremely potent effect on some microsomal enzymes, particularly glucuronyltransferase and benzpyrene hydroxylase, and that female rats may be more susceptible to TCDD actions than males. Marked increases in hepatic enzyme activity in female rats was observed following a single oral dose of 0.2 μg TCDD/kg (LD_{50} = 100 μg/kg) (glucuronyltransferase + 157%, benzpyrene hydroxylation + 683%), and the levels of induction increased with dose so that after 5.0 μg TCDD/kg glucuronyltransferase was induced 500% and benzpyrene hydroxylation 1400%. TCDD also increased cytochrome P-450, cytochrome b_5, and aniline hydroxylation, whereas oxidative demethylations of aminopyrine, ethylmorphine, and benzphetamine were decreased. NADPH cytochrome c reductase and β-glucuronidase were unaffected by any TCDD dose. Time–course studies revealed that increases reached a plateau 3 days after TCDD treatment following an initial lag period. Increased levels were maintained at the day 3 values through day 16 after which time activities began to return to normal although effects were still evident 38 days after treatment. Subfraction of microsomes into SER and RER revealed that TCDD markedly decreased SER to RER ratios in all parameters tested. There was no biochemical or histologic evidence of hepatotoxicity nor any effects on oxidative phosphorylation rates in liver mitochondria. These studies indicate that hepatic microsomal enzymes are extremely sensitive to TCDD body burdens.

Needs for Further Research

Our studies to date have served only to characterize the effects of TCDD on hepatic microsomal enzymes following animal exposures. The need for future research related to TCDD-microsomal interactions are many, and the following represents a list of some of the most urgent research needs: (1) study microsomal effects of *in vitro* addition of TCDD and related compounds directly to the incubation medium; (2) compare inductive properties of TCDD with 3-methylcholanthrene and other inducers; (3) determine structural requirements for induction by using structural analogs of TCDD as effectors of microsomal enzymes; (4) determine if TCDD induces extrahepatic microsomal enzymes and determine levels of induction in species other than the rat; (5) determine if induction of microsomal enzymes is related to increased synthesis, decreased degradation, or membrane effects; (6) determine rates of *in vivo* metabolism and excretion of test compounds, including steriods, following TCDD treatment; (7)

determine if maternal exposure of TCDD induces fetal enzymes; and (8) study possible relationships between TCDD induction of microsomal enzymes and teratogenic effects.

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